IMPORTANCE OF QUALITY OF CATTLE FEED

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Quality control in the compound feed industry not only involves the verification of quality standards established for each feed ingredient as it is received into storage in the mill, but also involves the close monitoring of the quality of ingredients through the period of storage prior to usage and during its processing. Quality control continues as ingredients are merged during the mixing process and as they finally go into storage as compound feed.

The efficiency of feed utilization in the livestock and the development of livestock feed industry of a country is dependent upon the quality of feeds. The quality of compounded feeds is never above the quality of its constituents as the quality of compounded feeds is governed by its constituents. A quality feed would supply all nutrients in adequate quantity of proportion with high digestibility. Intentional contamination / adulteration of feed ingredients to earn unethical and illegal profits seriously affect feed quality and thereby animal productivity and health.

In oil seed cakes category, soybean, groundnut, mustard, linseed, sesamum and sunflower are used in cattle and poultry feeds. Sometimes, other cakes such as cottonseed and copra are also used as main protein ingredient. Most compounded feeds contain limited amount of grains and oilseed cakes. Processing though mostly improve the quality but over and under processing coupled with long storage of ingredients before processing deteriorate the feed quality significantly.

In India the quality control is regulated by to a statuary body Bureau of Indian Standards (BIS). It was established under BIS Act, 1986. Bureau has set up subcommittee on animal feeds called Animal Feeds Sectional Committee has been specifically set up to establish the specifications of animal feeds and feed ingredients and are published as BIS specification. The approved published standards are revised from time to time. BIS is also responsible for publishing various methods of analysis of nutrients and anti nutritional factors present in animal feed as BIS standards/specifications. In Punjab, Director Dairy Development is concerned about the quality of cattle feeds through ñPunjab Compounded feed order 2006ò.

Quality Control

The purpose of quality control of raw materials is to ensure that minimum contract specifications are met. More precisely, it provides knowledge concerning the exact composition of raw materials and the levels of toxic substances normally present so that mixed feeds of the required nutritive value can be safely processed from them. These specifications are usually determined by a team consisting of the nutritionist, management personnel, and the quality control manager. The specifications relate to nutrient quality, cost, and the quality desired in the feedstuffs. Once the specifications are decided upon, they must be used and adhered to. Such a decision must be based on realistic reasoning, since it is useless to have specifications which are so narrow as to make purchasing impossible, or are so broad as to present severe problems for diet formulation.

The objective of quality control of feedstuffs is to ensure that a consumer should obtain feeds that are unadulterated, true to their nature and produce desired results. Quality control is therefore, defined as the maintenance of quality at levels and tolerances acceptable to the buyer while minimizing the cost of processing.

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Quality commitment and points to evaluate

To organize an in-plant quality control program, an overview of the total operation is the primary consideration; and the development of a quality control manual is logical first step as a useful guide to action. As an employee training tool, and as a reference for all company personnel. A typical quality control manual wills usually the following:

- An index or outline of content
- A statement of the companyôs quality control philosophy
- In-plant quality control supervisory and operator duties and responsibilities
- Sampling practices and procedures for ingredients and finished products
- A suggested ingredient assay schedule
- Laboratory report including interpretation as to their use
- Regulation and compliance (Good Manufacturing Practices)
- Production record keeping and procedures
- Package weight control, labeling, and coding
- Complaint procedures
- Product recall procedures
- Rework material guidelines
- Housekeeping (sanitation)requirements
- Ingredient purchasing specifications
- Warehousing and pest control practices
- Shelf-life and finished product turnover standards
- Guidelines for medicated feed manufacturing and handling
- Plant formula guidelines/standard operating practices for the handling of new and old formulas.
- Employee training in quality control
- In-process sampling, testing method, and test equipment for particle size reduction, batching and mixing, pellet quality , etc
- Maintenance practices and responsibilities
- Assignment of one person for total coordination of the program. The person should be given clear authority to articulate conditions and problems to management and should not be restricted in that by purchasing , production, sales, or any other person.
- or function
- All plant personnel, including delivery personnel, should be involved in the program and trained to perform their , individual quality control duties.
- All quality control stations-receiving; the various processing locations such as grinding, mixing ,pelting, and others; and bulk load out-should be provided with the necessary test equipment, forms for recording test results, sample bags, and other supplies.
- Periodic, routine compliance inspections should be conducted by appropriate management personnel using checklist to ascertain that the companyôs quality commitment standard are being met; and the results of those inspections should be shared with all levels of management as well as with plant sand truck fleet employees.

Common Adulterants in Feeds and Fodders

Adulteration is defined as the admixture of a pure substance with some cheaper and low quality substance. It is done intentionally usually to make money. In costly feed ingredients like oil seed cakes and feeds of animal origin like fish meal, adulteration is done by spraying urea in order to raise their protein content. However, sometimes brans, molasses are also added. Besides urea, oilseed cakes are adulterated with husk, non edible oilseed cakes.

Table 1. Common Adulterants and Anti nutritional Factors in some cereals and byproducts.

Ingredient	Adulterants	ANFs	Checks
Maize	Cobs, cob dust , sand	Aflatoxin	Freshness, colour, moisture, Mouldy odour, weevils.
Bajra	Sand, Old seed	T2 toxin, Zeralenone, NSPs	Freshness, colour, size moisture, Mouldy odour, weeds, sand silica.
Jowar	Sand, Old seed	T2 toxin, Zeralenone, NSPs and Tannin	Freshness, colour, size moisture, sand.
Ragi	Sand	NSPĉs, Mycotoxins	Freshness, colour, size, sand
Rice Kani	Sand , Bran , Husk, Marble, grit	Aflatoxin	Freshness, colour, Mouldy odour, sand, husk,rancid odour,
Wheat	Weed, Seed, Husk, Sand	Aflatoxin, NSPs	Freshness, Mouldy odour, Sand, Husk, Weed seeds.
Rice polish	Rice bran, Husk, Saw dust, Sand	Aflatoxins	Moisture, rancidity, Coarseness, Oiliness, Odour.
De oiled rice bran	Sand, Husk, Saw dust.	Aflatoxin	Moisture, Smell, roughness, Clumps.
Wheat bran	Ground rice husk, saw dust.		
Molasses	water		

Table 2. Common Adulterants and Anti nutritional Factors in some protein supplements

Ingredient	Adulterants	ANFs	Checks
Soybean	Sand and silica, Hulls, urea, raw soybeans	Aflatoxin, Trypsin inhibitor	Freshness, moisture, clumps, odour, colour, mould growth.
GNC	Hulls, sand, cheaper oil seeds, urea	Aflatoxins	Freshness, moisture, clumps, odour, colour, mould growth.
DMC	Hulls, sand, *Argimona maxicana* seeds, fibrous feed ingredients, urea.	Aflatoxins, Glucosinolates	Moisture, clumps, moulds growth.

Sunflower cake	Hulls, sand, castor seeds	Aflatoxin B1, T2 toxin	Freshness, Moisture, Odoutr, rancidity, clumps, mould growth.
Dry Fish/ Fish Meal	Sand , urea , Salt and other marine products, NPN	Gizerosine	Moisture, oil , sand, other marine products.
Meat and Bone Meal	Sand, Leather meal	Moisture, odour, colour	Biogenic amines, microbial contaminations

Table 3. Common Adulterants in some mineral supplements

Ingredient	**Adulterants**	**Checks**
Calcite	Sand, Magnesium	Moisture, colour, coarseness
DCP	Sand, Fluorine	Moisture, colour, odour
Mineral Mixture	Common salt, marble powder, sand, lime stone Magnesium	Moisture, colour, odour
Shell Grit	Sand	Colour, Uniformity

Quality Control of Feeds and feed ingredients

Quality control specifications of various feed ingredients and compound feeds laid down by BIS ensures to meet the minimum contract specifications, suitable for inclusion in the compounded feeds and indicating the maximum proportions of inclusion of feed stuffs.

Table 4. Quality Control of feed ingredients

Ingredient Quality (Qualitative)	Physical characteristics (analystôs skills): Color, Texture, Odour and Taste, Particle size (screen analysis), shape, Adulteration, damage and deterioration, bulk density, storage pests, feacal material, hair etc, spot chemical tests.
Ingredient Quality (Quantitative)	**Chemical analysis:** Moisture, CP, CF, EE, NFE, ash, Acid insoluble ash (silica or sand), salts, free fatty acids, biogenic amines urea, and NPN, amino acids. Anti-nutritional factor: **Extrinsic (contaminants):** mycotoxins, weeds, insecticide, herbicides, fungicides **Intrinsic:** allergins, lectins, phytoestrogens, glucosinolates (rape seed), saponins, tannins, ricin, sinapine, gossypol, (cotton seed cake), lipoxygenase, trypsin inhibitor, urea. Decomposition and rancidity test: acid value, peroxide value, etc. **Protein quality:** protein solubility or dispersibility, Nitrogen solubility,

	mailard reaction product, dye binding, pepsin digestibility, amino acid digestibility.

SAMPLING OF FEEDS

In India, BIS has laid down the following procedure and precautions for collecting the samples for analysis.

General requirements:

- In drawing, preparing, storing and handling samples, care should be taken that the properties of feeds are not affected.
- Take samples at a protected place not exposed to damp air, dust or soot.
- The sampling instrument shall be clean, dry and sterile when used.
- Protect the samples, the sampling instrument and the containers for samples from adventitious contamination.
- Preserve the samples in clean, dry and sterile containers. The sample containers shall be of such a size that they are almost completely filled by the sample.
- Each container shall be sealed air-tight with a stopper or a suitable closer after filling in such a way that it is not possible to open and reseal it without detection. Market full details of sampling i.e. the date of sampling, batch or code number, name of the manufacturer and other important particulars of the consignment.
- Samples shall be stored in such a manner that there is no deterioration of the material.
- Sampling shall be done by in the presence of the purchaser (or his representative) and the vendor (or his representative).

Sampling procedures for feed analysis: In all cases, at least 10% of the packages should be sampled. A minimum of approximately 1kg should be collected from each load. All cores should be combined in an airtight container. In India Bureau of Indian standards has laid down the following procedure of sampling the feeds.

Pierce (1985) recommended as follow:

Packages/bags	Number to be Sampled
1-10	1-3
11-25	2-4
26-50	3-6
51-75	6-8
76-100	8-10

All the containers in a single consignment of the material drawn from a single batch of manufacture shall constitute a lot. If a consignment is declared to consist of different batches of manufacture, the batches shall be grouped separately and the containers in each group shall constitute a separate lot.

- Samples shall be tested for each lot for ascertaining conformity of the material to there requirements of the specification.
- The number of containers to be selected from the lot shall depend on the size of the lot and shall be in accordance with col 1 and 2 of Table

Number of containers to be selected for sampling

Lot Size	Number of Containers to be selected
(N)	(n)

(1)	(2)
2 to 15	2
16 to 50	3
51 to 100	4
101 to 150	5
151 to 300	7
301 and above	10

- The containers shall be chosen at random from the lot and for this purpose a random number table as agreed to between the purchaser and the vendor shall be used (seen IS 4905: 1968).

Arrange all the containers in the lot in a systematic manner and starting from any container count 1, 2, 3,é é ..etc, up to r and so on. Every *r*th container shall be with drawn from the lot to give a sample for test where r =N/n , r being the integral part of N/n; where N is the total number of containers in the lot , and n the number of containers to be selected according to Table 2. If comes out to a fractional number, its value shall be taken to be as equal to its integral part.

TEST SAMPLES AND REFEREE SAMPLES

Preparation of Individual Samples: Draw with an appropriate sampling instrument equal quantities of the material from different parts of each container selected according to Table 2. The total quantity of the material drawn from each container shall be not less than 1.5 kg. Mix all the portions of the material drawn from the same container thoroughly. Take out about 0.75 kg of material and divide into three equal parts . Each portion, thus obtained shall constitute the test sample representing that particular container and shall be transferred immediately to clean and dry sample containers and sealed air-tight. These shall be labeled with particulars given under B-1.5.The individual samples obtained as above shall be formed into three sets in such a way that each set has test sample representing each container selected. One of the sets shall be for the purchaser, another for the vendor and the third for the referee.

Preparation of Composition Sample: From the mixed material from each selected container remaining after the individual samples have been taken, equal quantities of material from each container shall be taken and mixed up together so as to form a composite sample weighing not less than 0.75 kg. This composite sample shall be divided into three equal parts labeled with the particulars given under and sealed air-tight. One of these samples shall be for the purchase, another for the vendor and the third for the referee.

Referee Sample: Referee sample shall consist of a set of test samples and composite samples, and shall bear the seal of the purchaser and the vendor and shall be kept at a place agreed to between the two.

Testing of Samples: Samples shall be tested for each lot for ascertaining the conformity of the material to the requirements of this standards.

Criteria for Conformity: A lot shall be considered as conforming to the specification when the test results on the individual samples satisfy the requirement.

Evaluation of Feed for Quality

The feeds are usually subject to following 3 types of tests :

1. Physical
2. Chemical
3. Biological

Physical Evaluation: Physical evaluation is easy but rough in nature. One must be highly trained to identify the changes in the nature of the raw material/feeds.

Colour: The appearance of the ingredient will reveal its quality. Any deviation from normal colour of the feed ingredients gives an indication of something wrong. The maturity of the grain, storage conditions, presence of toxins, and contamination due to sand, possible use of insecticides/fungicides can be the factors affecting the colour. Orange to red colour of sorghum indicates high tannin content. Black coloured fish meal indicates the rancidity of fish oils.

Size: Size of the grains govern its energy value due to the proportional decrease/ increase in seed and its coat. Smaller the grain lower will be the ME value due to more proportion of coater hulls. To evaluate the cereals weight of a fixed number of grains usually 100 grains or fixed volume is taken. Higher weight indicates a higher ME value. This technique is called Test Weight.

Homogeneity: The presence of contaminants like other grains, husks broken grains, weed seeds, presence of feather, rat or bird excreta infested seeds is viewed. In the oil seed cakes closer observation will reveal the presence of fibrous material, especially in de-oiled groundnut cake, the cake with hulls which contains nearly 20 to 25% crude fibre can be visually identified. Rice polish is contaminated with husk. Clumps in mineral ingredients are not suitable for premixing.

Smell:- Smell is the next best indicator just standing near the stock itself will immediately indicate any difference in the normal smell. Musty Odour indicates the beginning of fungal contamination or boring insects. To detect rancidity in oil rich feed ingredients this is the best method. Odour of petroleum products is suggestive of excessive pesticide or fungicides. Leathery smell of meat meal indicates its adulteration with leather meal.

Taste:-Each ingredient has a different taste, any change in the taste like bitterness in the grains, soya, sunflower oil meal and groundnut cake indicates the presence of mycotoxins. The level of salt can be detected by tasting the ingredient and the feed. Bitter taste of rice polish indicates the rancidity of the fatty acids. But it is not advisable as certain very toxic and harmful substances may be present in it.

Touch:- Feeling the raw material will indicate the dryness. Chilliness indicates high moisture content. Clumps can be found out by inserting the hand inside the bag, The clumps may be due to high moisture content, improper storage, packing of fresh warm solvent extracted meal. Which crumble on application of light pressure. Clumps formed due to excess of moisture will be very hard. To evaluate rice polish, place about 25g of rice polish on the palm and close the fingers tightly and then open the fingers the rice polish will become like a solid mass if the crude fiber level is below 12% if the fiber level is high the mass will disintegrate once the fingers are opened.

Sound: - Dry grains on pouring down or biting will produce sound of spilling coins. The Common contaminant or adulterant is husk or sand. Winnowing is the best method to detect husk in the feedstuff. Sieving can be done to differentiate contaminants based on particle size. To detect for the presence of sand a weighed quantity of the grain is soaked in water then by sieving with hand the grains can be separated. The remaining water if decanted the settled sand can be weighed and the level of contamination can be assessed.

Chemical Evaluation: Analyse the feeds for proximate principles. This indicates possible constraints on usage due to the presence of excessive content of crude fibre, fat or total ash. Low CP and high CF of oil seed meals are indicative of adulteration with fibrous material. The high CF alone is indicative of adulteration with urea and or some inferior quality oil seed meals like mahua, castor or karanja cake.

The amount of acid insoluble ash is a good guide to the amount of sand or other dirt which may be present. The fish meals are usually adulterated with sand during drying process.

It is also desirable to determine the free fatty acid content of oily materials as this will affect palatability due to rancidity of oils. The chemical composition/specifications of various animal feeds are laid down by the BIS which act as guidelines for the suppliers, buyers and the users at farm level. The protein meals should also be analysed for their amino acid contents. NIR (Near Infrared Spectroscopy) is also a quick method to determine the proximate principles. Mobile van of Dairy Development Department Punjab provides the facility to the farmers.

4. **Biological Evaluation :** Biological evaluation of the feeds involve the use of animals, specialized persons to conduct the digestion and metabolism trails on the various species of livestock and poultry. These methods are time consuming.

Ingredient Specifications

Ingredient specifications are essential in a feed quality assurance program. Specifications serve as the basis from which purchasing agreements are written, feed/rations are formulated and ingredient inspections are performed. Ingredient description and general nutritional specifications may be found in BIS specifications for feeds and feed ingredients in India.

Toxins in animal feed: The various feed ingredients should be analyzed for the toxins present in them. Which are other wise injurious to the health of animals? The examples of toxins in the various feeds are given below:

1. Gossypol in cotton seed
2. Halmagglutinins in soybean and castor beans
3. Glucosinolates in rape seed
4. Tannins in sorghum, oil seed meal, mango seed kernel, mustard oil cake and lucerne meal
5. Cyanogenic glycosides in linseed and cassava
6. Phytic acid in all cereals, oilseed meals
7. Mycotoxins, primarily aflatoxins in maize, groundnut cake, etc.

Ultra violet screening is used whereby a greenish yellow fluorescence is observed when the sample is exposed to ultra violet light to detect mycotoxins. The maximum permissible levels of aflatoxins is depleted in the Table.

One should get from the best source of supply and one should have some idea of normal levels of toxicity which may be expected.
Fish meal, meat meal and bone meal should be checked for pathogenic bacteria like Salmonella.
Microscopic Evaluation of Animal Feed: Feed microscopy is commonly used for confirming the adulteration and identifying the adulterants. Feed ingredients, adulterants and contaminants must be studied under low and high magnification for distinguishing features whether coarsely or finely ground. At physical characteristics such as shape, color, and particle size, softness, hardness, and texture of the feeds are examined at low magnification of 8x to 50x. It is useful method to identify impurities/contaminants and evaluating the quality of feed ingredients. It also serves as a useful method for identifying missing ingredients in finished feed.

The plant cells and structural features of the feeds are observed at high magnification of 100xto 500 x since there characters are retained after grinding or even after powdering the feed ingredients.

A feed scientist must be familiar with of feed ingredients and adulterants and must have a collection of pure feed ingredients, adulterants and contaminants for the accurate and fast quality assurance results. The mashed and sieved feed should be used for a clearer observation of plant histology and microscopic appearance, heat the feed with 8% KOH steam bath for 30-45 min. if this treatment is not satisfactory, treat the fresh portion for a short time by gently heating with acidified chloral hydrate glycerol solution.

BIS specifications of Cattle feed

S.No.	Characteristic	Requirement		
		Type I	Type II	Type III
1.	Moisture, percent by mass, Max	11	11	11
2.	Crude protein (N x 6.25), percent by mass, Min	22	20	18
3.	Crude fat, percent by mass, Min	4.0	2.5	2.0
4	Crude fibre, percent by mass, max	10	12	15
5.	Acid insoluble ash, percent by mass, Max	3.0	4.0	5.0
6.	Salt (as NaCl), percent by mass, Max	1.0	1.0	1.0
7.	Calcium (as Ca), Percent by mass, Min	0.8	0.8	0.8
8.	Total phosphorus, percent by mass, Min	0.5	0.5	0.5
9.	Available phosphorus, percent by mass, Min	0.25	0.25	0.20
10.	Urea, percent by mass, Max	1.0	1.0	1.0
11.	Vitamin A, I.U.kg, Min	7000	7000	7000
12.	Vitamin D_3, I.U.kg, Min	1200	1200	1200
13.	Vitamin E, I.U/kg, Mim	30	30	30
14.	Aflatoxin B_I (ppb), Max	20	20	20

FEED COMPOSITION TABLE (DRY MATTER BASIS) (BIS)

S. No.	Feed Stuff	DM	ME (kcal /kg)	CP	CF	EE	NFE	Ca	P	Copper (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Sodium (per)	Zinc (mg/kg)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
ANIMAL (Scientific name not used)														
1.	Blood meal	88.8	1420	73.4	0.7	-	-	0.32	0.31	10.9	4132	5.8	0.35	-
2.	Bone meal	95.5	1044	14.6	2.5	3.1	5.6	27.00	12.11	17.2	884	32.0	0.48	447.0
3.	Fat	97.9	7700	-	-	97.9	-	-	-	-	-	-	-	-
4.	Meat meal	92.5	2319	56.2	2.2	11.9	8.7	2.68	2.06	10.4	471	10.2	1.80	-
5.	Meat and bone meal	94.0	2111	53.8	2.3	-	-	11.25	5.39	1.6	532	13.1	0.78	104.3
6.	Liver residue meal	90.9	3000	65.4	1.3	15.8	11.9	0.54	1.35	96.4	680	9.5	-	-
7.	Bajra - grain	89.6	2642	12.7	2.2	4.9	78.2	0.13	0.72	-	-	-	-	-
8.	Barley grain	96.4	2618	12.0	6.5	2.8	74.5	0.29	0.63	8.5	60	18.3	0.02	17.2
9.	Coconut - cake (2)	91.0	1190	22.6	12.5	8.7	49.4	0.23	0.66	20.1	2108	59.6	0.04	-
10.	Cottonseed - cake (2)	92.3	1556	25.9	25.4	8.6	33.7	0.52	0.86	20.7	319	22.9	0.04	-
FISH Scientific name not used)														
11.	- meal (2)	93.8	1834	43.1	3.6	4.3	11.5	7.16	1.67	21.7	320	38.9	0.19	-
12.	Groundnut - cake (2)	91.5	2596	40.9	8.9	7.9	36.4	0.23	0.59	-	-	27.7	-	-
13.	- cake, deoiled (2)	93.3	2328	48.6	11.2	2.2	27.2	0.31	0.67	-	-	31.5	0.07	21.7
14.	- plant meal (4)	92.1	1812	14.4	32.5	3.1	37.4	1.69	0.34	-	-	-	-	-
15.	Grains - Brewers dehydrated (2)	92.0	2732	28.2	16.3	-	-	0.29	0.54	23.2	272	40.9	0.28	-
16.	- distillers dehydrated (2)	91.6	-	31.8	12.6	-	-	0.22	0.60	23.5	284	37.8	0.05	-
17.	Guar Meal (2)	89.7	-	42.0	10.9	6.2	35.1	0.54	0.70	-	-	-	-	-
18.	Jowar Grain (1)	87.3	2645	10.3	3.6	4.6	78.1	0.18	0.32	15.8	-	16.3	0.04	15.4
19.	Linseed - cake (2)	90.7	1671	29.6	11.1	10.4	42.6	0.48	0.98	29.0	187	43.3	0.12	-
20.	Lucerne - leaf meal, dehydrated (4)	91.0	1.777	19.1	21.6	2.8	42.1	1.83	0.45	10.7	494	31.2	0.10	17.2
21.	Maize grain (1)	89.5	9309	9.2	2.4	3.9	82.8	0.25	0.40	4.0	30	4.8	0.01	12.1
22.	- gluten feed (2)	92.3	3315	26.9	5.1	4.8	59.6	0.15	0.28	-	-	-	-	-
23.	- gluten meal (2)	90.3	2705	49.9	2.0	4.2	41.4	0.22	0.35	31.0	440	8.0	0.11	-

24.	- grits byproduct (1)	90.8	2742	13.6	5.9	2.1	76.1	0.31	0.15	16.1	66	16.1	0.44	-
25.	Mohwa - residue (2)	-	-	14.5	30.9	5.4	31.0	-	-	-	-	-	-	-
26.	Mustard - cake (2)	91.3	2373	35.1	8.2	14.1	33.4	0.89	1.78	64.1	-	-	-	-
27.	Oats Grain (1)	91.7	2848	14.7	13.5	4.6	60.8	0.11	0.41	6.6	79	42.9	0.07	-
28.	penicillin mycelium waste (2)	91.8	-	31.9	8.4	6.7	34.5	3.97	1.12	-	-	-	-	-
29.	- residue (2)	-	-	17.9	5.6	5.5	35.8	3.80	0.17	-	-	-	-	-
30.	Poultry byproduct meal	93.0	-	56.4	0.9	17.8	7.6	3.95	1.73	-	-	-	-	-
31.	- feather meal (2)	91.1	-	94.6	0.4	2.5	10.2	0.65	0.32	-	-	-	-	-
32.	- manure, dehydrated (2)	93.5	791	25.7	15.2	2.5	31.2	9.86	1.42	-	-	-	-	-
33.	Rice kani (1)	90.7	2345	7.9	1.4	1.7	87.1	0.11	0.48	-	-	-	-	-
34.	- polish (1)	91.8	2937	12.7	11.2	13.9	48.6	0.27	1.37	-	-	-	-	-
35.	- polish deoiled (1)	92.9	2235	14.1	13.8	1.7	53.4	0.37	1.80	-	-	-	0.12	-
36.	Safflower cake decorticated	92.8	-	42.2	8.5	8.2	32.2	0.40	0.51	-	-	-	-	-
37.	cake, undccorticated	95.0	-	23.1	29.9	5.5	36.9	-	-	-	-	-	-	-
38.	Salseed cake, deoiled	90.4	3096	10.4	3.4	2.9	79.6	0.24	0.16	-	-	-	-	
39.	Sesame (Til, Gingelly)	90.7	1882	39.1	4.7	9.3	34.3	2.46	1.42	-	-	51.6	0.04	107.5
40.	Silkworm pupae meal, deoiled (2)	90.5	3000	69.8	3.9	2.2	15.5	0.29	0.58	-	-	-	-	-
41.	Soybean cake (2)	89.9	2694	41.7	6.3	21.2	26.0	0.36	0.90	20.0	178	35.9	0.27	-
42.	Molasses	73.6	2400	2.8	-	-	86.3	1.51	0.66	79.5	253	56.3	-	-
43.	Sunflower- cake (2)	89.1	2230	37.2	11.6	10.9	32.6	0.43	1.14	-	-	24.6	-	-
44.	Sunhemp - meal (2)	91.1	-	40.0	9.3	5.4	40.3	0.47	0.68	-	-	-	-	-
45.	Tapioca - flour (1)	-	3000	2.9	10.9	0.7	77.0	0.58	0.12	-	-	-	-	-
46.	- waste (2)	90.4	-	4.1	15.9	1.5	72.5	0.58	0.19	-	-	-	-	-
47.	Terramycin - waste (2)	96.6	-	31.7	7.2	4.1	15.4	-	-	-	-	-	-	-
48.	Triticale - grain (1)	92.9	3846	15.1	5.2	3.4	72.4	-	-	-	-	-	--	-
49.	Wheat bran	88.9	1069	14.7	11.3	3.8	62.3	0.19	1.12	13.8	100	130.0	0.07	-
50.	grain (1)	89.8	3045	10.3	2.1	2.6	82.3	0.18	0.43	10.8	56	57.0	0.07	15.6
51.	Yeast sludge	94.8	-	32.1	1.8	1.3	46.3	6.05	0.41	-	-	-	-	-

METHODS OF ANALYSIS

Moisture: Weigh and place 4-5 g of the sample in a covered, flat, aluminium dish. Dry to constant weight at 100-105°C in a drying oven.

$$\text{Moisture content (\%)} = \frac{\text{Weight fresh sample - Weight dry sample}}{\text{Weight fresh sample}} \times 100.$$

Crude Protein (Kjeldahl Method)

Reagents:
(a) sulphuric acid (98%), nitrogen free,
(b) potassium sulphate, reagent grade,
(c) mercuric oxide, reagent grade,
(d) paraffin wax,
(e) sodium hydroxide, 40% solution,
(f) sodium sulphide, 4% solution,
(g) pumice chips,
(h) boric acid/indicator solution. Add 5 ml of indicator solution (0.1% methyl red and 0.2% bromocresol green in alcohol) to 1 litre saturated boric acid solution, and

(i) hydrochloric acid standard solution (0.1N).

Apparatus: (a) macro Kjeldahl digestion and distillation units,
(b) Kjeldahl flasks (500 ml capacity or larger), and
(c) conical flasks, 250 ml.

Method: Accurately weigh 1 g of sample into a digestion- flask. Add 10 g potassium sulphate, 0.7 g mercuric oxide (pre-measured catalyst tablets containing these two reagents are available), and 20 ml sulphuric acid. Heat the flask gently at an inclined angle until frothing subsides and then boil until the solution clears. Continue boiling for an additional half hour. If the frothing is excessive, a small amount of paraffin wax may be added.

On cooling, add about 90 ml distilled water, recool, add 25 ml sulphide solution, and mix. Add a small piece of boiling chip to prevent bumping and 80 ml of sodium hydroxide solution while tilting the flask so that two layers are formed. Connect rapidly to the condenser unit, heat, and collect distilled ammonia in 50 ml boric acid/indicator solution. Collect 50 ml of distillate. On completion of distillation, remove the receiver (wash condenser tip) and titrate against standard acid solution.

Calculation:

Nitrogen content of sample (%)

$$= \frac{\left(\text{ml acid} \times \text{normality of standard acid}\right)}{\text{wt of sample (g)}} \times 0.014 \times 100$$

4.3 Crude Fat

Reagents and equipment:

(a) petroleum ether (b.p. 40-60°C),
(b) extraction thimbles, and
(c) Soxhlet extraction apparatus.

Method: Weigh into an extraction thimble 2-3 g of the dried sample (residue from dry matter determination can be used). Place the thimble inside the Soxhlet apparatus. Connect a dry pre-weighed solvent flask beneath the apparatus and add the required quantity of solvent and connect to condenser. Adjust the heating rate to give a condensation rate of 2 to 3 drops/s and extract for 16 h. (The extraction time may be reduced to a minimum of six h by increasing the condensation rate.) On completion, remove the thimble and reclaim ether using the apparatus. Complete the removal of ether on a boiling bath and dry flask at 105°C for 30 min. Cool in a desiccator and weigh.

Calculation:

Crude fat (% of DM)

$$= \frac{\text{weight of fat}}{\text{weight of sample}} \times \frac{100}{1}$$

Free fatty acids

Reagents and apparatus:

(a) ethyl alcohol,
(b) phenolphthalein (1% soln, in alcohol),
(c) sodium hydroxide (0.25N), and
(d) stoppered flasks, 250 ml. Method:

Weigh oil or fat into a stoppered flask and add 50 ml alcohol previously neutralised by adding sufficient 0.25N sodium hydroxide to give a faint pinkish colour with phenolphthalein (2 ml). Titrate with sodium hydroxide and vigorous shaking until a permanent faint pink colour appears.

Calculation:

Free fatty acids % (as oleic acid)

$$= \frac{\text{g oil or fat}}{7.05} \times \text{volume of 0.25 N NaOH used in titration}$$

Retain extracted sample for crude fibre analysis and extracted fat for free fatty acid determinations.

Crude Fibre

Reagents:

(a) sulphuric acid solution (0.25 N),
(b) sodium hydroxide solution (0.313N),
(c) antifoam reagent (Octyl alcohol),
(d) ethyl alcohol, and
(e) hydrochloric acid, 1% v/v.

Apparatus:

(a) beakers, 600 ml tall-sided,
(b) round-bottom flask condenser unit,
(c) Buchner flasks, 1 litre,
(d) Buchner funnels. Hartley 3 section pattern,
(e) crucibles, silica with porous base, and
(f) rubber cones to fit above.

Method: Weigh about 2 g of the dried, fat-free sample into a 600 ml beaker. Add 200 ml of hot sulphuric acid, place the beaker under the condenser, and bring to, boiling within 1 min. Boil gently for exactly 30 min, using distilled water to maintain volume and to wash down particles adhering to the sides. Use antifoam if necessary. Filter through Whatman No. 541 paper in a Buchner funnel, using suction, and wash well with boiling water. Transfer residue back to beaker and add 200 ml hot sodium hydroxide solution. Replace under the condenser and again bring to boil within 1 min. After boiling for exactly 30 min, filter through porous crucible and wash with boiling water, 1% hydrochloric acid and then again with boiling water. Wash twice with alcohol, dry overnight at 100°C, cool, and weigh. Ash at 500°C for 3 h, cool, and weigh. Calculate the weight of fibre by difference.

Calculation:

Crude fibre (% of fat-free DM)

$$= \frac{(\text{weight crucible} + \text{dried residue}) - (\text{weight crucible} + \text{ashed residue})}{(\text{weight of sample})} \times 100$$

Ash

Weigh a 2 g sample into a dry, tared porcelain dish and then place in a muffle furnace at 600°C for 6 h. Cool in a desiccator and weigh.

Calculation:

Ash (%)

$$= \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

Acid soluble and insoluble ash Reagents and apparatus:

(a) hydrochloric acid (1-2.5 v/v),
(b) filter paper, ashless, and
(c) dishes, porcelain.

Method: Use the residue obtained from the ash determination. Boil with 25 ml hydrochloric acid, taking care to avoid spattering, filter through ashless filter paper, and wash with hot water until acid free. Place filter paper and residue into a dry, tared porcelain dish and place in a muffle furnace at 600°C for 2 h or until carbon free.

Calculation:

Acid insoluble ash (%)

$$= \frac{\text{weight of acid - treated ash}}{\text{weight of sample}} \times 100$$

Calcium Reagents:

(a) hydrochloric acid (1-3 v/v),
(b) nitric acid (70%),
(c) ammonium hydroxide (1-1 v/v),
(d) methyl red indicator (dissolve 1 g in 200 ml alcohol),
(e) ammonium oxalate (4.2% solution),
(f) sulphuric acid (98%), and
(g) standard potassium permanganate solution (0.05 N).

Apparatus:

(a) porcelain dishes,
(b) volumetric flasks, 250 ml,
(c) beakers, 250 ml,
(d) quantitative filter paper and funnels, and
(e) burette.

Method: Weigh 2.5 g of finely ground material into a porcelain dish and ash as above (alternatively use residue from ash determination). Add 40 ml hydrochloric acid and a few drops of nitric acid to the residue, boil, cool, and transfer to a 250 ml volumetric flask. Dilute to volume and mix.

Pipette a suitable aliquot of the solution (100 ml for cereal feeds, 25 ml for mineral feeds) into a beaker, dilute to 100 ml and add 2 drops of methyl red. Add ammonium hydroxide one drop at a time until a brownish orange colour is obtained, then add two drops of hydrochloric acid to give a pink colour. Dilute with 50 ml water, boil, and add while stirring 10 ml of hot 4.2 percent ammonium oxalate solution. Adjust the pH with acid to bring back pink colour if necessary. Allow precipitate to settle out, and filter, washing precipitate with ammonium hydroxide solution (1.50 v/v). Place the filter paper with precipitate back in beaker and add a mixture of 125 ml water and 5 ml sulphuric acid. Heat to 70°C and titrate against the standard permanganate solution.

Calculation:

Calcium (%)

$$= \frac{\text{ml permanganate solution}}{\text{wt sample}} \times \frac{\text{aliquot used (ml)}}{250} \times 0.1$$

4.5.2 Phosphorous Reagents:

(a) Molybdovanadate reagent
Dissolve 40 g ammonium molybdate $4H_2O$ in 400 ml hot water and cool. Dissolve 2 g ammonium metavanadate in 250 ml hot water, cool, and add 450 ml 70 percent perchloric acid. Gradually add the molybdate solution to the vanadate solution with stirring and dilute to 2 litres.

(b) Phosphorous standards

Prepare stock solution by dissolving 8.788 g potassium dihydrogen orthophosphate in water and making up to 1 litre. Prepare the working solution by diluting the stock 1 in 20 (working concentrate 0.1 mg P/ml).

Apparatus:

(a) spectrophotometer to read at 400 m , and
(b) graduated flasks, 100 ml.

Method: Pipette an aliquot of the sample solution prepared as for the calcium determination into a 100 ml flask and add 20 ml of the molybdovanadate reagent. Make up the volume, mix, and let stand for 10 min. Transfer aliquote of the working standard containing 0.5, 0.8, 1.0, and 1.5 mg phosphorus to 100 ml flasks and treat as above. Read sample at 400 my setting the 0.5 mg standard at 100 percent transmission. Determine mg phosphorus in each sample aliquot from a standard curve.

Sodium Chloride

Reagents:

(a) standard 0.1 N silver nitrate solution,
(b) standard 0.1 N ammonium thiocyanate solution,
(c) ferric indicator - saturated aqueous solution of ferric aluminium,
(d) potassium permanganate solution - 6% w/v,
(e) urea solution - 5% w/v, and
(f) acetone (A.R. grade).

Method: Weigh 2 g sample into a 250 ml conical flask. Moisten sample with 20 ml water and then add, by pipette, 15 ml 0.1 N silver nitrate solution and mix well. Add 20 ml concentrated nitric acid and 10 ml potassium permanganate solution and mix. Heat mixture continuously until liquid clears and nitrous fumes are evolved; then cool. Add 10 ml urea solution and allow to stand for 10 min. Add 10 ml acetone and 5 ml ferric indicator, and back titrate the excess silver nitrate with the 0.1 N thiocyanate solution to the red brown end point.

Calculation:

Calculate results as sodium chloride,

$$\% \, NaCl = \frac{(15.00 - ml\, 0.1N\, NH_4CNS, \times 0.585)}{g\, sample\, taken}$$

Molasses Analysis

Total sugars

Reagents

(a) Fehling's solution (Soxhlet modification)
(i) Dissolve 34 639 g of copper sulphate 5 H_2O in water and make up to 500 ml. Filter, and

(ii) Dissolve 173 g of Potassium sodium tartrate 4 H_2O and 50 g sodium hydroxide in water, dilute to 500 ml, stand for two days, and filter through prepared asbestos.

(b) Invert sugar standards :Prepare stock solution by adding 5 ml of hydrochloric acid (sp.g 1.18) to 9.5 g of sucrose in solution and dilute to about 100 ml. After storing for two days at room temperature, dilute to 1 litre. Prepare working solutions (5 mg/ml) by pipetting 100 ml of the stock solution into a 200 ml volumetric flask, and neutralising with 20 percent sodium hydroxide using phenolphthalein as the indicator. Dilute to mark, and mix.

(c) hydrochloric acid (sp. g 1.18),
(d) hydrochloric acid (0.5 N),
(e) sodium hydroxide (20%),
(f) phenolphthalein indicator (1% solution in alcohol), and
(g) methylene blue indicator (1% aqueous solution).

Apparatus:

(a) electric heater, and
(b) conical flasks, 300 ml.

Method: Dissolve 8 g of liquid molasses and make up to 500 ml. Carry out an acid hydrolysis on 100 ml of the filtrate by adding 5 ml of hydrochloric acid (sp. g 1.18) and allow to stand for 24 h. Neutralise with sodium hydroxide (20 percent) using phenolphthalein as indicator, and then dilute to 200 ml.

Standardisation of Soxhlet solution. Pipette 10 ml of Soxhlet solutions (a) and (b) into a conical flask, mix, and add 30 ml of water. Add from a burette a volume of working standard that is almost sufficient to reduce the copper in the Soxhlet solution. Bring to boiling and continue boiling for two minutes. Add four drops of methylene blue and rapidly complete the titration, while still boiling, until a bright orange colour is resumed. Repeat several times and determine the volume of solution required to completely reduce 20 ml of the Soxhlet solution.

Titration of sample. First, carry out an approximate titration: pipette 10 ml of solutions (a) and (b) into a flask and add 10 ml aliquot of-the sample solution. Add 40 ml of water and bring to boil. If blue colour persists, titrate with a standard working solution and calculate the approximate sugar content of the sample.

To accurately determine the sugar content, pipette 10 ml of Soxhlet solutions (a) and (b) into a flask and add an aliquot of the sample solution. The volume of sample used will depend on the sugar content of the sample (see Table 1).

Table 1 Sample Volumes Used in Soxhlet Titration

ml H_2O	ml sample	g sample in aliquot	Total sugar as invert, %
40	10	0.08	73
35	15	0.12	82-58
30	20	0.16	61-41
25	25	0.20	49-35
20	30	0.24	41-29

(Reproduced from official Methods of Analysis of the AOAC, 1970)

Add water as indicated in the table, mix, and boil. During boiling, add a quantity of working standard from a burette so that the titration is nearly complete. Add methylene blue and complete the titration. Calculate the percentage sugar (as invert) by the formula:

$$\% \text{ sugar} = (F - M) \times 1 \times 100/W$$

where

F - is the volume of standard needed to reduce 20 ml of Soxhlet solution,
M - is the volume of standard sugar solution required to complete the back titration,
1 - is the weight of invert sugar in 1 ml of working standard, and
W - is the weight of sample in aliquot used.

Potassium

Reagents and equipment:
(a) hydrochloric acid (concentrated),
(b) potassium standard
To prepare stock solution (500 ppm K), dissolve 0.477 g potassium chloride (Analar) and make up to 500 ml with distilled water. To prepare working standard (10 ppm), dilute 1:50.
(c) silica crucibles,
(d) flame photometer, and
(e) muffle furnace.

Method: Dry 2 g of sample in a silica crucible at 100°C to expel moisture. Add a few drops of pure olive oil and heat over flame until swelling stops. Ash at 500°C in muffle furnace for 24 h, cool, and add 2 ml concentrated hydrochloric acid to dissolve the residue. Make up to 100 ml. Take 1 ml of this solution and make a further dilution to 100 ml.

Set the flame photometer to give a reading of 100 with the 10 ppm standard, and then read sample solution. If the sample reading does not fall between 50 and 100 make a fresh dilution to give an appropriate reading.

Anti-metabolite and Toxins in Feeds

Urease activity in soybean meal

Reagents:
(a) dimethylaminobenzaldehyde solution (DMAB)
Dissolve 16 g DMAB in 1 litre 95% ethyl alcohol, and add 100 ml concentrated hydrochloric acid (stable for one month).
(b) pyrophosphate buffer
Dissolve 23.3 g $Na_4P_2O_7$ in approximately 980 ml distilled water. Add 3 ml of concentrated HCl and then additional HCl until the pH of the buffer is 7.7-7.8. Dilute to 1 litre.
(c) buffered urea solution
Dissolve 04 g urea in 1 litre pyrophosphate buffer (stable for 1 week).
(d) zinc acetate solution
Dissolve 22.0 g zinc acetate $2H_2O$ in distilled water, add 3 ml of glacial acetate acid, and dilute to 100 ml.
(e) potassium ferrocyanide solution
Dissolve 10.6 g $K_4Fe\ (CN)_6$, $3H_2O$ in distilled water, and dilute to 100 ml.
(f) charcoal.

Apparatus:

(a) water bath at 40°C, capable of maintaining temperature within ± 1°C, with shaking device,
(b) conical flasks, 125 ml,
(c) volumetric flasks, 25 ml, and
(d) spectrophotometer.

Method: Accurately weigh 1 g of soybean meal into a conical flask and add 50 ml of the buffered urea solution. Incubate in water bath for exactly 30 min at 40 C with shaking. Remove from water bath and quickly add 0.5 ml each of concentrated HCl, ferrocyanide solution, zinc acetate solution, and 0.1 g of charcoal. Shake for 15 min and filter. If the filtrate is coloured, repeat the procedure using more charcoal. Pipette 10 ml aliquots of the filtrate and the DMAB solution into a 25 ml volumetric flask and make up to volume with distilled water. Make up also a reagent blank (10 ml DMAB made up to 25 ml with water) and a urea blank (10 ml buffered urea solution and 10 ml DMAB made up to 25 ml with water). Prepare a standard curve by pipetting aliquots of buffered urea solution from 2 to 12 ml into 25 ml volumetric flasks, adding 10 ml of DMAB and make up to volume.

Mix flasks well, stand in water bath at 25°C for 10 min, and then read at 430 m . Calculate urease activity as mg/litre urea in urea blank less mg/litre urea in sample.

Free gossypol in cottonseed meal

Free procedures are described for the determination of free gossypol: the first for normal meals, and the second for meals which have been chemically treated and contain dianilinogossypol.

Reagents:
(a) aqueous acetone, 7 parts acetone, 3 parts distilled water (v/v);
(b) Aqueous acetone - aniline solution
To 700 ml acetone and 300 ml distilled water add 0.5 ml redistilled aniline. Prepare solution daily.
(c) Aqueous isopropyl alcohol solution: 8 parts isopropyl alcohol, 2 parts distilled water (v/v),
(d) Aniline
Distill reagent grade aniline over a small quantity of zinc dust, discarding the first and last 10 percent of the distillate. Store refrigerated in a brown glass stoppered bottle. Stable for several months.
(e) Standard gossypol solution
(i) Dissolve 25 mg of pure gossypol in aniline-free acetone and transfer to a 250 ml volumetric flask using 100 ml of acetone. Add 75 ml of distilled water, dilute to volume with acetone, and-mix.

(ii) Take 50 ml of solution (a), add 100 ml pure acetone, 60 ml of distilled water, mix, and dilute to 250 ml with pure acetone. Solution (b) contains 0.02 mg gossypol/ml and is stable for 24 h in darkness.

Apparatus:

(a) mechanical shaker,
(b) spectrophotometer,
(c) conical flasks, 250 ml capacity,
(d) volumetric flasks, 25 and 250 ml, and
(e) water bath (boiling).

Method: Grind sample to pass 1 mm screen taking care not to overheat. Take approximately 1 g of the sample and add 25 ml of pure acetone. Stir for a few minutes, filter, and divide filtrate into two. To one portion add a pellet of sodium hydroxide and heat in a water bath for a few minutes. A light yellow extract which does not change colour with sodium hydroxide indicates that the cottonseed meal is untreated and procedure (1) should be used. A deep orange red colour in the tube containing sodium hydroxide indicates the presence of dianilinogossypol and this requires that procedure (2) be used.

Procedure (1): weigh 0.5 to 1 g of sample, depending on expected gossypol content, into a conical flask and add glass beads. Pipette in 50 ml of aqueous acetone solution, stopper the flask, and shake for one hour. Filter, discarding the first few ml of filtrate, and then pipette out duplicate aliquots into 25 ml volumetric flasks. (Take aliquots from 2 to 10 ml, again depending on expected gossypol content.) Dilute one of the aliquots to volume with aqueous isopropyl alcohol (Solution a) while to the other aliquot (Solution b) add 2 ml redistilled aniline; heat in a boiling water bath for 30 min together with a reagent blank containing 2 ml of aniline and a volume of aqueous acetone solution equal to the sample aliquot. Remove solution b and the blank, add sufficient aqueous isopropyl alcohol to effect homogeneous solution, and cool to room temperature in a water bath. Dilute to volume with aqueous isopropyl alcohol.

Read samples at 400 mu. Set instrument to 0 absorbance with aqueous isopropyl alcohol, and determine absorbance of solution a and reagent blank. If the reagent blank is below 0.022 absorbance proceed as below, otherwise repeat the analysis using freshly-distilled aniline.

Determine the absorbance of solution b, with the reagent blank set at 0 absorbance. Calculate the corrected absorbance of the sample aliquot: the corrected absorbance is the absorbance of solution b minus the absorbance of solution a. Determine the mg of free gossypol present in the sample solution using the calibration curve (see below).

Procedure (2): Weigh 1 g of sample into a conical flask, add 50 ml aqueous acetone, shake, and filter as above. Pipette duplicate aliquots of the filtrate (from 2 to 5 ml, depending on expected free gossypol level) into 25 ml volumetric flasks. Dilute one of the aliquots to volume (solution a) with aqueous isopropyl alcohol and leave for at least 30 min before reading on the spectrophotometer. Treat the other aliquot (solution b) as in procedure (1), determine the absorbances of solutions a and b as before, and calculate the apparent content of gossypol in both solutions a and b by using the calibration curve (see below).

Preparation of calibration curve: pipette duplicate 1, 2, 3, 4, 5, 7, 8, and 10 aliquots of the 0.02 mg/ml gossypol standard into 25 ml volumetric flasks. Dilute one set (solution a) to volume with aqueous isopropyl alcohol and determine absorbances as previously. To the other set (solution b) add 2 ml of redistilled aniline and proceed as previously. Prepare one reagent blank, using 2 ml aniline and 10 ml of aqueous acetone, heated together with the standards. Determine absorbances as in procedure (1) and calculate the corrected optical density for each standard solution:

Corrected absorbance = (absorbance solution b - absorbance solution a). Plot the standard curve, plotting corrected absorbance against gossypol concentrate in the 25 ml volume.

Calculate free gossypol percent in normal meals as:

$$\text{Free gossypol } \% = \frac{5G}{WV}$$

where

G - is the graph reading
W - sample weight
V - aliquot volume used

For chemically treated meals:

$$\text{Free gossypol } \% = \frac{5(B-A)}{WV}$$

where

A - mg apparent free gossypol in sample aliquot (a)
B - mg apparent free gossypol in sample aliquot (b)
W - sample weight
V - aliquot volume used

Thioglucoside determination

The method described will give approximate thioglucoside content but does not allow the individual thioglucosides and isothiocyanates to be determined.

Reagents and apparatus:

(a) barium chloride (5% solution),
(b) volumetric flasks, 600 ml, and
(c) steam bath.

Method: To 10 g meal (de-fatted by Soxhlet extraction) add 250 ml distilled water, hydrolyse at 54°C for 1 h and then boil for 2h, keeping volume constant. Filter, retaining filtrate, and wash residue three times with 50 ml hot water. Add washings to initial filtrate and make up volume to 600 ml. Precipitate barium sulphate by heating and adding excess barium chloride solution. Leave on a steam bath for a few hours and then filter. Ash in a muffle furnace and then weigh precipitate.

Calculate approximate thioglucoside content as:

$$\%\ \text{trioglucoside} = \frac{(\text{M. wt. trioglucoside})(\text{Wt. of } BaSO_4)}{(\text{M. wt. } BaSO_4)(\text{Sample Wt.})} \times 100$$

Aflatoxin analysis : A method of aflatoxin analysis is outlined below which is suitable for materials such as groundnut meal, coconut meal, and palm kernel meal. For full details of the method, and for alternative procedures reference should be made to Methods of Aflatoxin Analysis by B. D. Jones (1972), Report No. G70, Tropical Products Institute, London.

Reagents:

(a) chloroform (reagent grade),
(b) diethyl ether (reagent grade),
(c) chloroform/methanol mixture (95/5 v/v),
(d) "Celite", diatomaceous earth,
(e) Kieselgel 'G' (Merck),
(f) qualitative standard
Helps to distinguish aflatoxin spots from other fluorescent spots which may be present. A groundnut meal containing aflatoxins B, obtainable from the Tropical Products Institute, London, can be used for this purpose.

Apparatus: (a) thin layer chromatography plates, 20 X 20 cm,
(b) UV lamp, peak emission at 365,
(c) bottles, wide-mouthed, 250 ml,
(d) micropipettes, and
(e) shaking device.

Method: Weigh 10 ml of material into a wide mouthed bottle and thoroughly mix in 10 ml of water. (If high fat material is used, a prior Soxhlet extraction with petroleum ether will be necessary.) Add 100 ml of chloroform, stopper with a chloroform resistant bung, and shake for 30 min. Filter the extract through "Celite", take 20 ml of filtrate and make up to 25 ml (solution a). Take another 20 ml of filtrate and concentrate to 5 ml (solution b).

Prepare thin layer plates by shaking Kieselgel 'G' (100 g) with water (220 ml) for 20 min and apply the mixture to the plates with a suitable apparatus to a depth of 509 . Leave for 1 h, then dry at 100°C. Spot 10 and 20 of solution b, and 5 and 10 of solution a onto a plate, together with a qualitative standard spot, in a line 2 cm from the bottom of the plate and at least 2 cm in from each side. Carry out the spot application in subdued light.

Develop the plate in diethyl ether to a height of 12 cm. Allow to dry in subdued light then redevelop the plate in chloroform methanol (95/5, v/v) to a height of 10 cm from the baseline. Examine the plate in a dark room, 30 cm from the UV source. The presence of a blue fluorescent spot at Rf 0.5 to 0.55 indicates aflatoxin B (check that the standard spot also lies in this range). The presence of a second spot at Rf 0.45 to 5 indicates aflatoxin G. The toxicity level of a sample can then be classified in terms of aflatoxins B and G according to Table 2.